

# Clinical Significance of the Wild Type p53-Induced Phosphatase 1 Expression in Invasive Breast Cancer

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# Clinical Significance of the Wild Type p53-Induced Phosphatase 1 Expression in Invasive Breast Cancer

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## Abstract

The nuclear expression of wild type p53-induced phosphatase 1 (Wip1) protein was found to be positive in 21 patients (10.4%) out of 201 breast cancer patients in our study. The *protein phosphatase magnesium dependent 1 delta* DNA copy number was significantly correlated with Wip1 protein expression, which was positively correlated with p21 expression. Tumors with positive Wip1 expression and negative p21 expression showed the poorest prognosis of all tumors examined.

**Background:** Wild type p53-induced phosphatase 1 (Wip1), encoded by the protein phosphatase magnesium dependent 1 delta (*PPM1D*), inhibits p53. *PPM1D* amplification has been reported in breast cancer. Breast cancer can sometimes develop without a tumor protein 53 (*TP53*) mutation. In these cases, the p53 pathway might be disrupted by alternative mechanisms, and Wip1 is reported to be a key molecule involved. **Materials and Methods:** Primary invasive ductal carcinoma specimens were obtained from 201 cases, for which archival tissue samples for immunohistochemistry were available. We evaluated Wip1 and p21 protein expression (201 cases), *Wip1* mRNA expression (63 cases), *PPM1D* DNA copy number (71 cases) and *TP53* status (36 cases) using available samples among the 201 cases, and analyzed their relationships with clinicopathological factors and prognosis. **Results:** The nuclear expression of Wip1 protein was positive in 21 cases (10.4%). The *PPM1D* DNA copy number was significantly correlated with Wip1 protein expression. All cases with *PPM1D* amplification by single-nucleotide polymorphism comparative genomic hybridization array showed positive nuclear Wip1 expression. Wip1 protein expression was positively correlated with p21 expression. The tumors with positive Wip1 and negative p21 expression showed the poorest prognosis among all tumor types. **Conclusion:** The protein expression of Wip1 might be regulated by *PPM1D* amplification, independent of *TP53* status. Positive Wip1 and negative p21 expression was associated with the poorest prognosis and suggests the loss of p53 function.

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**Keywords:** Oncogene, p21, *PPM1D*, *TP53*, Wip1

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## Introduction

Wild type p53-induced phosphatase 1 (Wip1), a member of the serine/threonine protein phosphatases, is a key component in the DNA damage response (DDR) network.<sup>1,2</sup> Wip1 is encoded by the protein phosphatase magnesium dependent 1 delta (*PPM1D*), located on 17q23.<sup>3</sup> Wip1 inhibits p53 function via the dephosphorylation of p53 and several proteins in the DDR/checkpoint pathways, including ataxia telangiectasia mutated (ATM), checkpoint kinase 1 (Chk1), checkpoint kinase 2 (Chk2), murine double minute, and p38 mitogen activated protein kinase.<sup>1,2</sup>

Protein phosphatase magnesium dependent 1 delta gene amplification and/or Wip1 overexpression have been observed in numerous tumors, including breast cancer.<sup>3,4</sup> Several studies have

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reported that *Wip1* mRNA or Wip1 protein overexpression is associated with a poor prognosis in several cancers, such as non-small-cell lung cancer,<sup>5</sup> hepatocellular carcinoma,<sup>6</sup> and colorectal cancer.<sup>7</sup> However, univariate survival analyses have failed to show a consistent association between *PPM1D* gene amplification or Wip1 overexpression and the prognosis in breast cancer.<sup>1,8</sup> Bilal et al reported that gene amplification at several loci, including the *PPM1D* gene, was useful as an independent marker of a poor prognosis associated with early relapse in estrogen receptor (ER)-positive breast cancers treated with tamoxifen.<sup>9</sup>

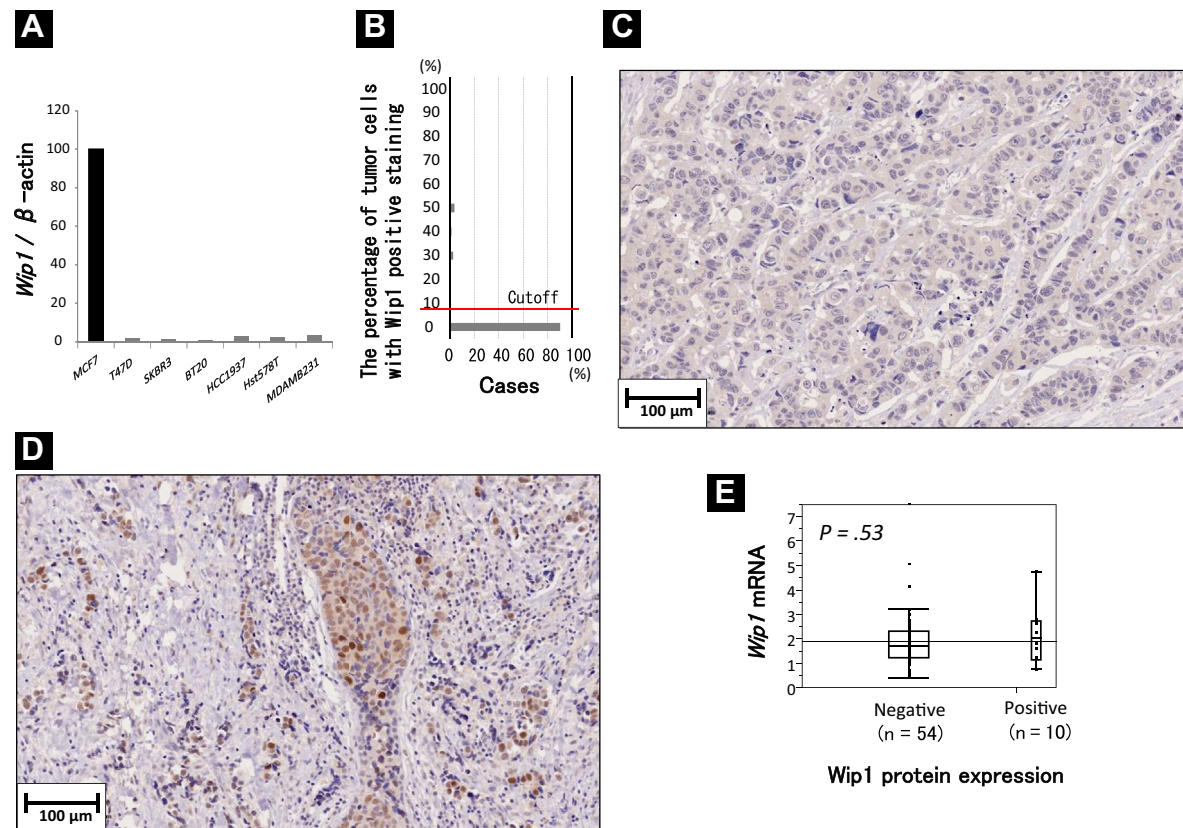
p53 is a master regulatory protein, encoded by the tumor protein 53 (*TP53*) gene, that is involved in diverse cellular metabolic processes such as apoptosis, DNA repair, and cell cycle arrest.<sup>10,11</sup> The protective function of p53 as a tumor suppressor is lost in >50% of human cancers, caused by mutations in the *TP53* gene.<sup>12</sup> In many other cancers, the p53 pathway might be disrupted by alternative mechanisms, and the lack of functional p53 protein induces cancer development and progression.<sup>13,14</sup> As its molecular function, p53

regulates the transcription of numerous genes,<sup>15,16</sup> which is controlled by post-translational modifications.<sup>17</sup> In particular, p53 can be classified into 2 different phosphorylation states: p53 arrester and p53 killer.<sup>18</sup> p53 arrester, phosphorylated at Ser15 and Ser20,<sup>17,19,20</sup> controls the cell cycle arrest module and induces the expression of p21.<sup>18</sup> p53 killer, phosphorylated at Ser15, Ser20, and Ser46,<sup>21,22</sup> controls the apoptotic module.<sup>18</sup> Wip1 induces the alteration of p53 killer to p53 arrester through the dephosphorylation of Ser46 and Wip1 is also activated by the p53 arrester itself.<sup>18,23</sup>

p21 is an important factor in cell cycle regulation and induces cell cycle arrest in the G1 phase.<sup>24,25</sup> Using MCF7, in which Wip1 as well as p21 are positive, Mirzayans et al reported that the inhibition of p21 leads to a decrease in Wip1 expression.<sup>26</sup> They suggested that p21 might contribute to the positive regulation of Wip1. However, the association between p21 and Wip1 in breast cancer is not fully elucidated.

Recently, Wip1 inhibitors have been reported in several studies.<sup>27-30</sup> The inhibition of Wip1 might have an important therapeutic role in suppressing tumor growth and evolution.<sup>27</sup> The

**Figure 1** Wild Type p53-Induced Phosphatase 1 (Wip1) Expression in Breast Cancer Cell Lines and Breast Cancer Tissue Samples. (A) Quantitative Reverse Transcriptase Polymerase Chain Reaction Data Show the Wip1 Transcription Level in Luminal Type (MCF7, T47D), Basal Type (BT20, HCC1937, Hst578T, MDA-MB231) and HER2 Type (SKBR3) Breast Cancer Cell Lines. The Graph Shows the Percentage of Tumor Cells With Wip1-Positive Staining Using Immunohistochemistry (B). Immunohistochemical Analyses for Wip1 in Breast Cancer Tissue Samples. Representative Images Showing a Low Expression of Wip1 (C) and a High Expression of Wip1 (D). The Association Between the Wip1 mRNA Expression and Wip1 Protein Expression in Breast Cancer Tissue Samples (E)



oral administration of Wip1 inhibitors in mice resulted in expected pharmacodynamic effects and caused the inhibition of lymphoma xenograft growth.<sup>27</sup> In addition, Wip1 inhibitor has been reported to potentiate the sensitivity to murine double minute inhibitors.<sup>29</sup> Wip1 is expected to be a novel molecular target for several cancers, including breast cancer.

Therefore, we supposed that Wip1 is an important factor to induce the loss of p53 function in breast cancer patients. Thus, we hypothesized that p53 function is indirectly predicted by evaluating Wip1 and p21 expression. In this study, we evaluated the expression of *Wip1* mRNA, Wip1 protein, *PPM1D* DNA copy number alteration, *TP53* status, and p21 expression. We also analyzed the relationship between Wip1 expression and clinicopathological features, prognosis, *TP53* status, and p21 expression of breast cancer patients to determine the clinical significance of Wip1.

## Materials and Methods

### Cell Lines, Culture Conditions, and Reagents

MCF7, T47D, MDA-MB231, HCC1937, HS578T, BT20, and SKBr3 cells were obtained from the American Type Culture Collection (Manassas, VA) and MCF7, MDA-MB231, HS578T, BT20 and SKBr3 were maintained in Dulbecco modified Eagle's medium (Mediatech, Manassas, VA) containing 10% fetal bovine serum (Life Technologies Japan, Tokyo, Japan) in 5% CO<sub>2</sub> at 37°C. T47D and HCC1937 cells were maintained in RPMI 1640 medium (Sigma, Tokyo, Japan) containing 10% fetal bovine serum (Life Technologies Japan) in 5% CO<sub>2</sub> at 37°C.

### Patient and Specimens

Primary invasive ductal carcinoma specimens were obtained from Japanese patients at stage I to III who underwent surgery in the Department of Surgery and Science, Kyushu University Hospital, between 2000 and 2006. Two hundred one cases, for which archival tissue samples for immunohistochemistry (IHC) were available were included in this study. No patients had received neoadjuvant chemotherapy or endocrine therapy, and histologically, special types were also excluded. The histological diagnosis was on the basis of the World Health Organization criteria. Written informed consent was obtained from all patients. The institutional review board of our university approved this study (27-197).

### Total RNA Isolation and Quantitative Reverse Transcriptase Polymerase Chain Reaction

The expression of *Wip1* mRNA was evaluated using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Sixty-three specimens, suitable for RNA extraction, were evaluated. Immediately after surgery, the specimens were placed in liquid nitrogen and stored at -80°C. Total RNA was extracted from each specimen with Isogen (Nippon Gene, Tokyo, Japan), and cDNA was synthesized from RNAs using Super Script III FirstStrand Synthesis Super Mix (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's recommendations. qRT-PCR was performed using a LightCycler 480 System II (Roche Diagnostics, Basel, Switzerland) and QuantiFast SYBR Green PCR kit (Qiagen, Hilden, Germany).  $\beta$ -Actin was used as an internal control. Human reference RNA (Promega, Madison, WI) was used as a standard for quantitation. The oligodeoxynucleotide primers used for Wip1 were

**Table 1** Association Between Expression of Wip1 Protein and the Clinicopathological Characteristics

Characteristic	Wip1 (n = 201)		P
	Negative (n = 180)	Positive (n = 21)	
<b>Age, Years</b> Mean $\pm$ SD, Range	53 $\pm$ 13 (26-85)	55 $\pm$ 15 (32-80)	.17
<b>Tumor Size, cm</b> Mean $\pm$ SD, Range	2.0 $\pm$ 2.3 (0.5-5.5)	2.4 $\pm$ 1.3 (1.2-6.0)	.96
<b>LN Metastasis</b>			
Negative	110 (64)	9 (47)	.21
Positive	61 (36)	10 (53)	
<b>Nuclear Grade</b>			
1, 2	125 (70)	15 (79)	.60
3	54 (30)	4 (21)	
<b>Ki-67, % Mean <math>\pm</math> SD</b>	15.4 $\pm$ 1.4	19.6 $\pm$ 4.7	.57
<b>ER</b>			
Negative	47 (28)	4 (19)	.60
Positive	123 (72)	17 (81)	
<b>PR</b>			
Negative	71 (41)	12 (57)	.24
Positive	101 (59)	9 (43)	
<b>HER2</b>			
Negative	95 (72)	12 (63)	.43
Positive	37 (28)	7 (37)	
<b>Subtype</b>			
HR <sup>+</sup> HER2 <sup>-</sup>	76 (58)	9 (47)	.06
HR <sup>+</sup> HER2 <sup>+</sup>	14 (11)	6 (32)	
HR <sup>-</sup> HER2 <sup>+</sup>	23 (18)	1 (5)	
HR <sup>-</sup> HER2 <sup>-</sup>	17 (13)	3 (16)	

Data are presented as n (%), except where otherwise specified.

Abbreviations: ER = estrogen receptor; HR = hormone receptor; LN = lymph node; PR = progesterone receptor; Wip1 = wild type p53-induced phosphatase 1.

as follows: forward, 5'-AGCAGAAGGGTTTCACCTCG-3' and reverse, 5'-CTGGCAGTTGTCCCTGATGT-3'. The primers used for  $\beta$ -actin were as follows: forward, 5'-GAAATCTGGCACCA-CACCT-3' and reverse, 5'-TAGCACAGCCTGGATAGCAA-3'.

### Evaluation of Estrogen Receptor, Progesterone Receptor, HER2, and Ki-67

The ER, progesterone receptor (PgR), and HER2 status were examined as previously reported.<sup>31</sup> ER and PgR were considered to be positive if  $\geq$  1% of the nuclei of cancer cells were stained on IHC. Tumors were considered to be HER2-positive only if they were scored 3+ in IHC or 2+ in IHC in combination with HER2 amplification (ratio > 2.0) using fluorescence in situ hybridization. Ki-67 was examined as previously reported.<sup>32</sup>

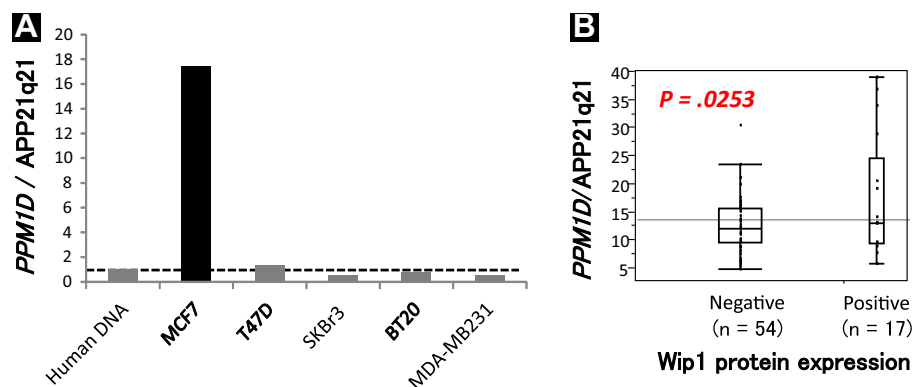
### Expression of Wip1 and p21 Protein

The expression of the Wip1 proteins and p21 were assessed using IHC. The primary antibodies used were as follows: Wip1 (anti-mouse monoclonal, sc-376257, 1:100; Santa Cruz Biotech, Dallas, TX), and p21 (anti-mouse monoclonal, WAF-1-L, 1:100;



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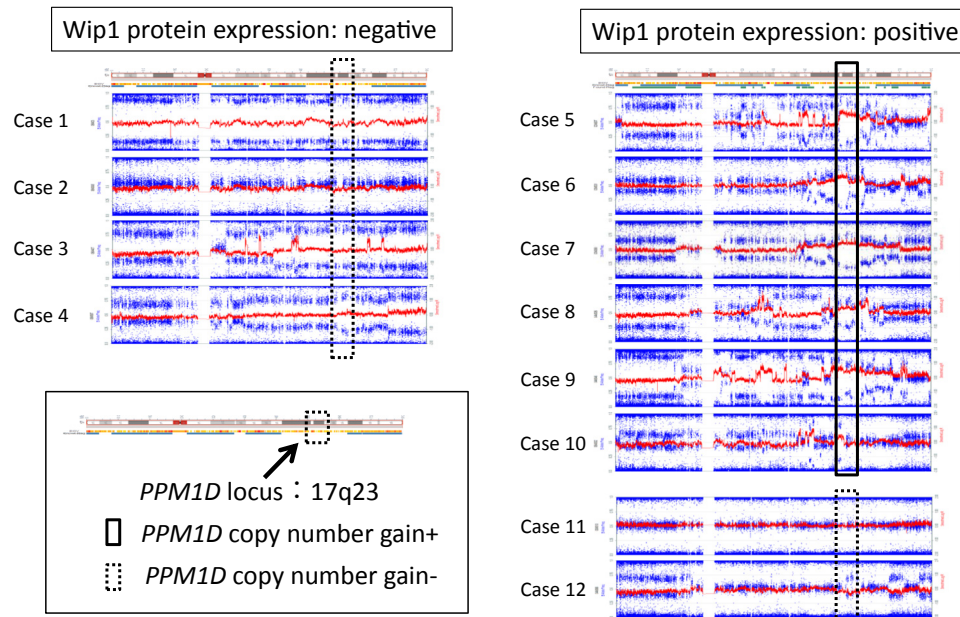
**Figure 2** Copy Number Alteration of *PPM1D* in Breast Cancer Cell Lines and Breast Cancer Tissue Samples. (A) Genomic Polymerase Chain Reaction Data Show Copy Number Alteration of *PPM1D* Locus Among Breast Cancer Cell Lines. (B) The Association Between the Expression of Wild Type p53-Induced Phosphatase 1 (Wip1) Protein and Copy Number Alteration of *PPM1D*



Leica, Tokyo, Japan). The sections from formalin-fixed, paraffin-embedded tissue specimens were used, and the sections were deparaffinized with xylene and rehydrated in graded alcohol. The sections were incubated in 10 mM citrate buffer (pH 9.0, S2367; Dako, Glostrup, Denmark) in an autoclave at 121°C for antigen retrieval for 10 and 15 minutes for Wip1 and p21, respectively. After quenching the endogenous peroxidase with 3% H<sub>2</sub>O<sub>2</sub> in methanol for 30 minutes, the sections were incubated with primary antibodies at 4°C overnight and then labeled with the Envision Detection System (Dako) for 1 hour at room temperature. The

sections were developed with 3,3'-diaminobenzidine tetrahydrochloride (DAB plus; Dako) and then counterstained with 10% Mayer hematoxylin, dehydrated, and mounted. Wip1 and p21 expression were independently evaluated by 2 researchers (Y.I. and N.Y.), who were blinded to the patients' clinical characteristics. Wip1 and p21 indices were determined as the percentage of tumor cells with positive nuclear staining among the counted tumor cells. Samples were classified as Wip1-positive if the Wip1 index was >10%. Ten percent was used as the cutoff according to the previous reports.<sup>33-35</sup> In our cohort, most of the cases showed nuclear

**Figure 3** Single Nucleotide Polymorphism Comparative Genomic Hybridization (CGH) Array Data Showing 6 of the 8 Wild Type p53-Induced Phosphatase 1 (Wip1)-Positive Cases Accompanied by *PPM1D* Locus Amplification



staining <10% (Figure 1B). Samples were classified as p21-positive if the p21 index was >5%, in accord with a previous report.<sup>36</sup>

### Genomic DNA Polymerase Chain Reaction

The *PPM1D* DNA copy number was analyzed using genomic polymerase chain reaction (PCR) in 71 cases, for which the extraction of the genomic DNA were available, including 17 Wip1 protein-positive and 54 Wip1 protein-negative cases. The amyloid precursor protein (*APP*) gene, located on locus 21q21, for which no amplifications in breast cancer have been reported, was used as the internal reference.<sup>37</sup> The primers used for *PPM1D* genomic PCR were forward: 5'-CCAAGGGTGAATTCTAAGGACC-3' and reverse: 5'-GGGTATGACTACACCTTGGAC-3'. The primers used for *APP* genomic PCR were forward: 5'-TCAGGTTGACGCCGCTGT-3' and reverse: 5'-TTCGTAGCCGTTCTGCTGC-3'.

### Single Nucleotide Polymorphism-Comparative Genomic Hybridization Array

The copy number analysis of the *PPM1D* gene was performed by whole genome single nucleotide polymorphism (SNP)-comparative genomic hybridization (CGH) array using genomic DNA from 12 breast cancer specimens including 8 Wip1 protein-positive cases and 4 Wip1 protein-negative cases. A tiling array was designed with a mean probe density of 1 probe per 1169 base pairs, 50-mer length, covering the whole chromosomal regions, including chromosome 17. Hybridizations were performed in the Nimble Gen Service Laboratory as described previously.<sup>38</sup>

### TP53 Gene Mutation Analysis

We analyzed the *TP53* gene mutation from 36 breast cancer specimens including 10 Wip1 protein-positive cases and 26 Wip1 protein-negative cases. The *TP53* gene, exon 5 to exon 9 including exon-intron junctions, was amplified using PCR with p53 primers (Nippon Gene) and Ex Taq DNA polymerase with 3' exonuclease activity (TaKaRa Bio Inc, Tokyo, Japan). The p53 primers were as follows: exon 5 forward, 5'-TGCAGGAGGTGCTTACACATG-3'; exon 5 reverse, 5'-TCCACTCGGATAAGATGCTG-3'; exon 6 forward, 5'-GAAAATCTGGCACCACACCT-3'; exon 6 reverse, 5'-GGAGGGCCACTGACAACCA-3'; exon 7 forward, 5'-TGCCACAGGTCTCCCAAGG-3'; exon 7 reverse, 5'-GCA-CAGCAGGCCAGTGTGCA-3'; exon 8-9 forward,

**Table 2** Wild Type p53-Induced Phosphatase 1 Protein Expression and *TP53* Mutation Status

Wip1, n (%)	TP53 Status		P
	Wild Type (n = 31)	Mutated (n = 5)	
Negative	22 (39)	4 (15)	.68
Positive	9 (90)	1 (10)	

Abbreviation: Wip1 = wild type p53-induced phosphatase 1.

5'-TTGGGAGTAGATGGAGCCT-3'; and exon 8-9 reverse, 5'-AGTGTTAGACTGGAACTTT-3'. The PCR products were purified and used as templates for cycle sequencing reactions with the Big Dye Terminator Cycle Sequencing Kit version 1.1 (Applied Biosystems, Foster City, CA). Mutations detected in a PCR product were verified using reverse sequencing and reconfirmed in 2 independently amplified PCR products.<sup>39</sup>

### Statistical Analyses

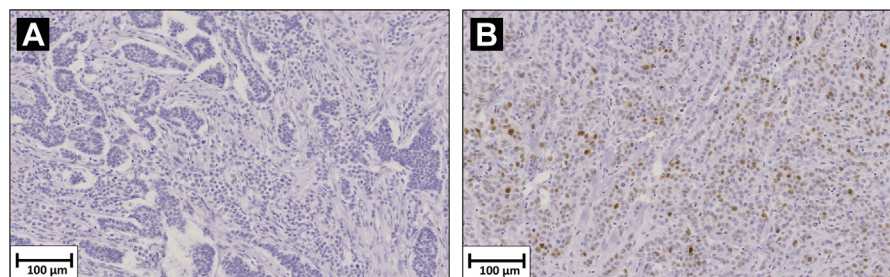
The statistical analyses were performed using the JMP software program, version 9.0.1 (SAS Institute Inc, Cary, NC). Relationships among the clinicopathological factors and Wip1 expression were analyzed using  $\chi^2$  tests, Fisher exact probability tests, and Student *t* test. Survival curves were plotted using the Kaplan-Meier method, and the log rank test was used to determine the associations between individual variables and survival. Recurrence-free survival (RFS) was defined as the time from surgery to the first breast cancer event, including local recurrence, distant metastasis, or a new cancer in the contralateral breast. Differences were considered to be statistically significant for values of  $P < .05$ .

## Results

### Wild Type p53-Induced Phosphatase 1 mRNA Expression in Breast Cancer Cell Lines and Breast Cancer Tissues

The *Wip1* mRNA expression evaluated using qRT-PCR was significantly elevated in the luminal type cell line (MCF7) and low in the other cells (T47D, MDA-MB231, HCC1937, HS578T, BT20, and SKBr3; Figure 1A). In contrast, there was no significant correlation between *Wip1* mRNA expression and prognosis (data not shown).

**Figure 4** p21 Expression in Breast Cancer Tissue Samples. Representative Images Showing (A) a Low Expression of p21 and (B) a High Expression of p21



# Clinical Significance of Wip1 Expression in Breast Cancer

**Table 3** Wild Type p53-Induced Phosphatase 1 Protein Expression and p21 Expression

Wip1, n (%)	p21		P
	Negative (n = 129)	Positive (n = 71)	
Negative	139 (77)	41 (23)	<b>.03</b>
Positive	11 (52)	10 (48)	

Positive Wip1 expression was significantly associated with positive p21 expression (shown in bold).

Abbreviation: Wip1 = wild type p53-induced phosphatase 1.

## Wild Type p53-Induced Phosphatase 1 Protein Expression in Breast Cancer Specimens

The Wip1 protein expression level was evaluated using IHC in breast cancer specimens. Cell blocks of MCF7 were used as positive controls. In the tumor cells, immunostaining for Wip1 was observed in the nucleus as well as cytoplasm. In this study, we evaluated Wip1 expression in the nucleus.<sup>33,40</sup> Representative photographs of Wip1-positive and Wip1-negative tumors are shown in Figure 1C and D. Positive Wip1 expression was observed in 21 cases (10.4%). There was no significant correlation between Wip1

mRNA expression and Wip1 protein expression (Figure 1E). There was no significant correlation between Wip1 protein expression and clinicopathological factors (Table 1).

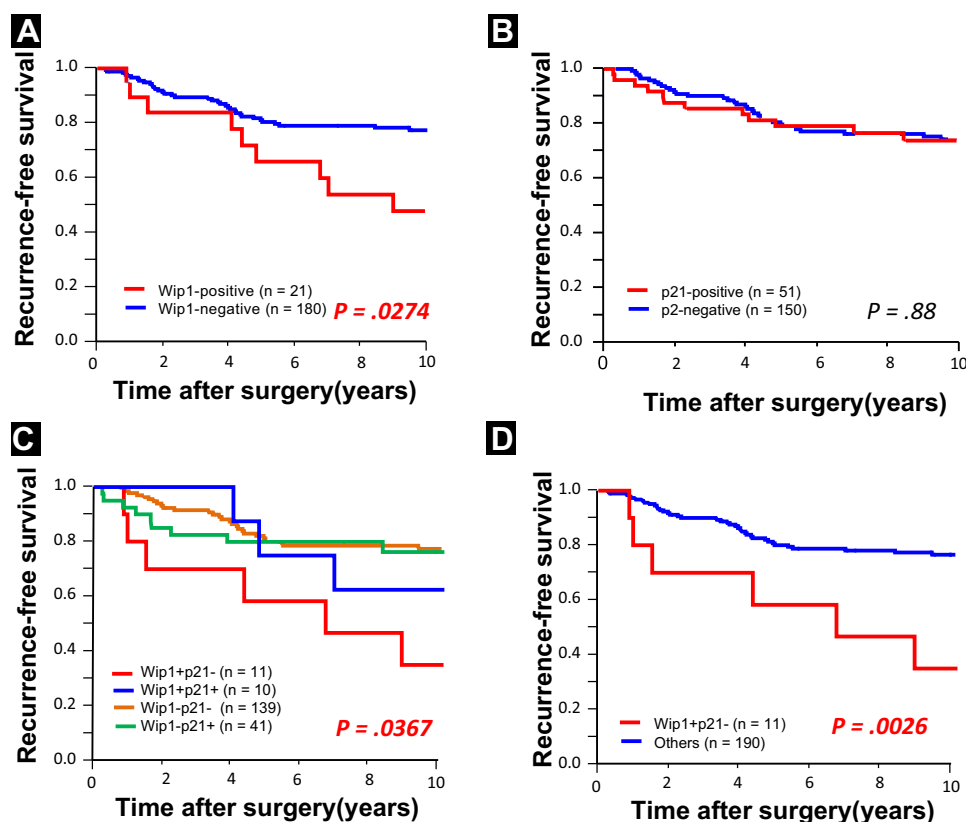
## Protein Phosphatase Magnesium Dependent 1 Delta DNA Copy Number Alteration in Breast Cancer Cell Lines and Specimens

The *PPM1D* DNA copy number was significantly elevated in MCF7 and low in the other cells (T47D, MDA-MB231, BT20, and SKBr3; Figure 2A). In breast cancer specimens, a positive correlation was found between *PPM1D* DNA copy number and Wip1 protein expression ( $P = .0253$ ; Figure 2B).

## Copy Number Alteration in the *PPM1D* Gene Locus Using SNP-CGH Array

We performed SNP-CGH array in 8 cases with positive Wip1 protein expression and 4 cases with negative expression to clarify potential mechanisms of disruption of the intact allele of the *PPM1D* gene in Wip1 protein expression. Significant amplification at locus 17q23, on which the *PPM1D* gene was encoded, was detected in 6 cases, all of which showed elevated Wip1 expression. No *PPM1D* amplification was observed in the cases with negative Wip1 expression (Figure 3).

**Figure 5** Kaplan-Meier Survival Curves of Recurrence-Free Survival for Breast Cancer Patients Stratified According to (A) Wild Type p53-Induced Phosphatase 1 (Wip1) Protein Expression, (B) p21 Protein Expression and (C) Wip1 and (D) p21 Expression. Positive Wip1 Expression and Negative p21 Expression Showed the Poorest Prognosis Among the Groups Examined



### Wild-Type p53-Induced Phosphatase 1 Protein Expression and TP53 Mutation Status

TP53 mutation status was analyzed using direct sequencing. Of the 10 cases with Wip1 protein expression, only 1 had a TP53 mutation, whereas another 10 cases had wild type TP53. In contrast, of the 26 cases with negative Wip1 protein expression, 4 had a TP53 mutation. However, there was no significant correlation between the Wip1 protein expression and TP53 mutation status (Table 2).

### p21 Protein Expression in Breast Cancer Specimens

p21 expression was investigated in 201 breast cancer specimens. Representative photographs of positive and negative p21 expression are shown in Figure 4. In tumor cells, immunostaining for p21 was observed in the nucleus. Positive p21 expression was observed in 51 cases (25.4%). Positive Wip1 expression was significantly associated with positive p21 expression ( $P = .03$ ; Table 3). There was no significant correlation between Wip1 and p21 expression and clinicopathologic characteristics (data not shown).

### Wild Type p53-Induced Phosphatase 1 and p21 Protein Expression and Prognosis

Wild type p53-induced phosphatase 1 protein expression was significantly associated with a poor prognosis in terms of RFS ( $P = .0274$ ; Figure 5A). However, there was no significant correlation between p21 expression and RFS (Figure 5B). In the combination analysis of Wip1 and p21 expression, Wip1-positive and p21-negative cases showed a significantly shorter RFS than the other groups (Figure 5C and D).

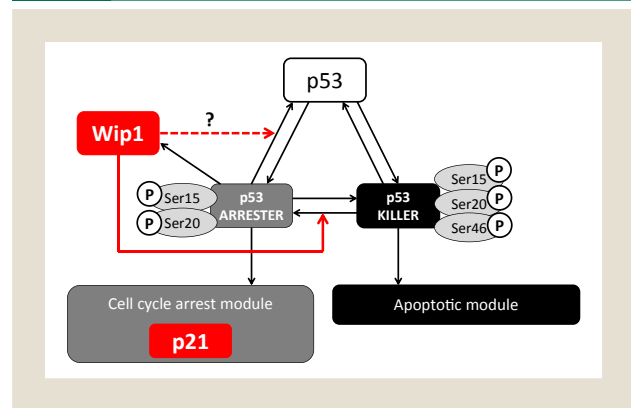
## Discussion

There is increasing evidence that *PPM1D* is an oncogene.<sup>18,41</sup> Wip1, the transcriptional product of *PPM1D*, exists mainly in the nucleus, and dephosphorylates several proteins in the DDR/checkpoint pathways.<sup>1,2</sup> Therefore, Wip1 might play an important role as an oncogene in the nucleus. To our knowledge, this is the first report to show that Wip1 nuclear protein expression is associated with *PPM1D* amplification in invasive breast cancer using genomic PCR and SNP-CGH.

Wild type p53-induced phosphatase 1 nuclear protein expression is considered to be regulated by *PPM1D* amplification. However, in some cases with Wip1 protein expression, *PPM1D* gene amplification was not observed. According to previous reports, the amount of Wip1 protein in tumors is much higher than the transcript levels, suggesting additional mechanisms for regulating the Wip1 protein expression level, including post-translational modifications, the regulation of protein degradation, and post-transcriptional mechanisms like alternative splicing.<sup>42-44</sup>

In cancers with wild type TP53, the p53 pathway is disrupted by several mechanisms,<sup>14,15</sup> and Wip1 is reported to be a key molecule involved.<sup>1</sup> The relationship between *PPM1D* amplification and TP53 status has been inconsistent. In 1 study, most of breast cancers with *PPM1D* amplification were reported to show no structural changes in the TP53 gene.<sup>4</sup> However, in The Cancer Genome Atlas studies, TP53 mutations have been reported in breast cancer samples with *PPM1D* amplification or Wip1 overexpression.<sup>41</sup> Demidov et al reported that *PPM1D*

**Figure 6** Schematic Illustration of the Possible Association Between Wild Type p53-Induced Phosphatase 1 (Wip1) and the Phosphorylation (P) States of p53. The Molecular Function of p53 is Controlled by Phosphorylation. p53 is Classified as Either p53 Arrestor or p53 Killer According to Its Phosphorylation States. p53 Arrestor, Phosphorylated at Ser15 and Ser20, Controls the Cell Cycle Arrest Module and Induces the Expression of p21. p53 Killer, Phosphorylated at Ser15, Ser20, and Ser46, Controls the Apoptotic Module. Wip1 Induces p53 Killer to p53 Arrestor Alteration Via Ser 46 Dephosphorylation. In Some Cases, Wip1 Dephosphorylates Ser15 and Ser20 Through Several Pathways and Might Lead to the Loss of the p53 Function



amplification or Wip1 overexpression was not dependent on TP53 status in vivo.<sup>45</sup> In our study, of the 10 cases with Wip1 protein expression, only 1 had a TP53 mutation.

Our data show that positive Wip1 expression was significantly associated with positive p21 expression. Wip1 induces p53 killer to p53 arrestor alteration via Ser 46 dephosphorylation and p53 arrestor induces the expression of p21 and controls cell cycle arrest.<sup>18</sup> This previous report supports the positive correlation between Wip1 and p21 expression in this study. However, some cases showed positive Wip1 expression without p21 expression. One potential reason for this apparent discrepancy is that Wip1 can also dephosphorylate Ser15 and Ser20 of p53 arrestor through several pathways, such as ATM and Chk2 (Figure 6).<sup>17-21,46</sup>

In the present study, Wip1 protein expression was significantly associated with a poor RFS. Furthermore, the expression pattern of positive Wip1 and negative p21 showed the worst prognosis among the groups examined; this outcome might be because of the loss of the p53 function among these populations (Figure 6).

## Conclusion

Wild type p53-induced phosphatase 1 protein expression in the nucleus was significantly associated with the *PPM1D* DNA copy number gain and p21 expression. In addition, the tumors with positive Wip1 expression and negative p21 expression showed the poorest prognosis among the evaluated cohort. The Wip1 protein expression might be regulated by *PPM1D* amplification, independent of TP53 status. Positive Wip1 and negative p21 expression might reflect the loss of p53 function.



# Clinical Significance of Wip1 Expression in Breast Cancer

## Clinical Practice Points

- Some breast cancers can develop without a *TP53* mutation. In such cases, the p53 pathway might be disrupted by alternative mechanisms. One of the key molecules involved in these mechanisms is Wip1, which works as an oncogene dephosphorylating several proteins including p53.
- The *PPM1D* DNA copy number was significantly correlated with the Wip1 protein expression. Amplification at the *PPM1D* locus was detected using SNP-CGH array, and all of the cases showed positive nuclear Wip1 expression. To our knowledge, this is the first report to show a significant association between the nuclear Wip1 protein expression and *PPM1D* amplification in breast cancer tissues.
- Wild type p53-induced phosphatase 1 protein expression in the nucleus was significantly associated with p21 expression. The tumors with positive Wip1 and negative p21 expression showed the poorest prognosis among the groups evaluated. Positive Wip1 and negative p21 expression suggests the loss of p53 function.

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## Disclosure

The authors have stated that they have no conflicts of interest.

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